

Abstract of the Disclosure

The present invention relates to a method of sequencing a target polynucleotide by enzymatic and/or chemical means. The sequencing method includes a method for characterizing multiple alleles in a sample, a method of calculating confidence levels in ascertained sequences, a method for comparing polynucleotide sequences, and a method of resolving ambiguities in a polynucleotide sequence. It also provides methods for appropriately preparing samples, for immobilizing template molecules, for organizing the template molecules, and to conduct the sequencing of many molecules in parallel. The method involves analyzing molecules as members of an array. Many target polynucleotides or many segments of a single target polynucleotide can be sequenced simultaneously. In a preferred embodiment, the method involves analyzing individual molecules within an array and base calls are based on the signals from two or more molecules. A method to prevent non-specific signal in sequencing is also provided. The invention is readily automated, both for small-scale and large-scale operation and relevant algorithms and the composition of kits and systems are provided.